An Optimal Number of Molecules for Signal Amplification and Discrimination in a Chemical Cascade

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ABSTRACT Understanding the information processing ability of signal transduction pathways is of great importance because of their crucial roles in triggering various cellular responses. Despite continuing theoretical investigation, some important aspects of signal transduction such as a transient response and its connection to stochasticity originating from a small number of molecules have not yet been well understood. It is, however, through these aspects that unexpected and nontrivial properties of the information processing emerge. In this article, we analyze the transient behavior of a simple signaling cascade by taking into account the stochasticity originating from the small number of molecules. We identify several properties of the signaling cascade that emerge as a result of the interplay between the stochasticity and transient dynamics of the cascade. We specifically demonstrate that each step of the cascade has an optimal number of signaling molecules at which the average signal amplitude becomes maximal. We further investigate the connection between a finite number of molecules and the ability of the cascade to discriminate between true and error signals, which cannot be inferred from deterministic descriptions. The implications of our results are discussed from both biological and mathematical viewpoints.

INTRODUCTION

Recent advances in systems biology have shed new light on the dynamical aspects of intracellular phenomena (1–4). The dynamical aspects of signal transduction pathways and their information processing capabilities are of particular interest due to their roles in triggering dynamic cellular responses (5,6).

Intracellular signal transduction pathways are often viewed as combinations of several common motifs of signal transduction, such as the MAP kinase cascade (7). It is therefore important to elucidate the properties of each motif, including input-output relations for various types of inputs and parameter dependencies of these relations.

Mathematical analysis has contributed to clarifying such properties (8–10). The mathematical modeling of signal transduction pathways was initiated by the pioneering work of Goldbeter and Koshland three decades ago (11,12). Since then, various mathematical models have been proposed. Some focus on modeling and analyzing specific pathways (13–16). Others attempt to elucidate general properties in an abstract model of signaling cascade (17–24). Overall, properties such as sensitivity and specificity were used to evaluate the performance of the pathways (9,11,18).

Most of the models, however, are devoted to the analysis of the stationary responses to constant input signals (11,12, 17,18,21,25). As a result, relatively little is known about the transient dynamics of signaling pathways. Pioneering work in this area was carried out by Heinrich et al. (20), who conducted extensive theoretical analysis on the transient behaviors of signal transduction pathways from a deterministic viewpoint. They investigated the relation between the transient dynamics of the cascade and various structures such as feedback interactions and crosstalk with other pathways, which appear prominently in actual cells.

However, transient dynamics of signal transduction pathways has not been studied well from the stochastic viewpoint. The experimental evidence for stochasticity in intracellular reactions is now rapidly accumulating (26–30). The stochasticity originating from a small number of molecules has been attracting particular interest, and the impact of such stochasticity in genetic networks has been intensively investigated (31–38). This stochasticity may also considerably influence intracellular signal transduction because the signaling molecules, such as enzymes and transcription factors, are proteins whose numbers are often small in a cell. For example, a dendritic spine of a Purkinje cell has a volume of $10^{-19}(\mathrm{m}^3)$ and so 1 μM of a chemical corresponds to 60 molecules, and 100 nM corresponds to six molecules (39).

Nevertheless, only a few studies have been conducted on the potential effects of stochasticity on the performance of intracellular signal transduction pathways (40–44). Furthermore, the scope of these few studies was restricted to the influence of stochasticity on the stationary responses of pathways to constant inputs. However, it can be through the interplay of two dynamical aspects of pathways, transient behavior and stochasticity, that unexpected and nontrivial properties of the information processing functions emerge.

In this article, we analyze the transient behavior of a simple signaling cascade by taking into account the stochasticity originating from small numbers of molecules. From our

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analysis we identify several properties of the signaling cascade that emerge as a result of the interplay between the stochasticity and the transient dynamics of the cascade.

This article is organized as follows. In the next section, we show the details of the signaling cascade to be analyzed in this article. Then, deterministic and stochastic models of the cascade, a method for their numerical calculation, and several characteristics for transient dynamics are introduced. Finally, we examine the transient responses of the signaling cascade to binary inputs by focusing on the dependence on the number of the signaling molecules, the input intensity, and the cascade step.

We demonstrate using numerical simulations the emergence of two novel properties of the signaling cascades: signal amplification and the discrimination between true and error signals. We further demonstrate that the appearance of these properties depends strongly on the number of the signaling molecules, the number of cascade steps, and the input intensity.

MODEL AND METHODS

A signaling cascade

A schematic diagram of the signaling cascade we investigate in this article is shown in Fig. 1. The cascade is composed of M signaling molecular species whose numbers are assumed to be constant and equal to N for all steps of the cascade.

Each signaling molecule has two states, inactive and active. Inactive molecules in the $(i + 1)^{\text{th}}$ step are catalytically activated by active ones in the i^{th} step, and the molecules in the first step are activated by the input to the cascade. The activated molecules at each step become spontaneously inactive.

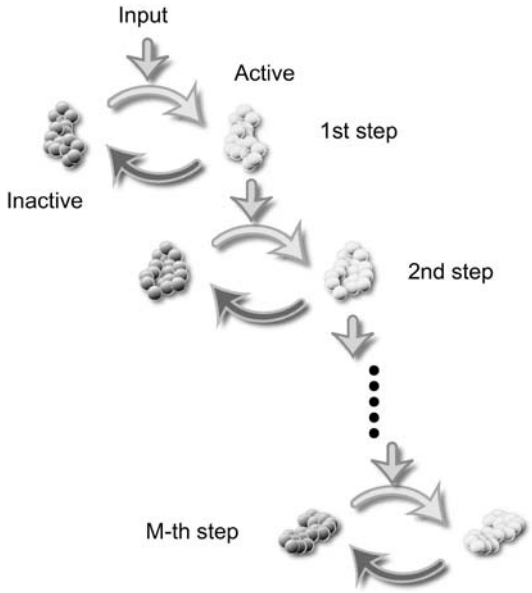

FIGURE 1 Schematic diagram of the signaling cascade. The input signal activates the inactive signaling molecules of the first step. The activated signaling molecules of the i^{th} step catalytically activate the inactive signaling molecules of the $(i + 1)^{\text{th}}$ step. The activated molecules of each step spontaneously become inactive.

The number of active molecules in the i^{th} step is designated by $n_i \in [0, N]$, while $x_i(t) = n_i(t)/N$ represents the ratio of active molecules in the i^{th} step.

When the number of molecules is large enough, the dynamics of the cascade can be approximately described by the following deterministic rate equations:

$$\frac{dx_1(t)}{dt} = k_{f_1}(1 - x_1(t))I(t) - k_{b_1}x_1(t),$$
$$\frac{dx_i(t)}{dt} = k_{f_i}(1 - x_i(t))x_{i-1}(t) - k_{b_i}x_i(t), \quad (i \geq 2), \qquad (1)$$

where $I(t)$ is the input, k_{f_1} and k_{b_1} are the rates of the activation and inactivation reactions in the first step of the cascade, and k_{f_i} and k_{b_i} $(i \geq 2)$ are those of the i^{th} step. The term $(1 - x_j(t))$ $(j \geq 1)$ is the ratio of inactive molecules in the j^{th} step, which results from a conservation law of the total number of molecules in each cascade step.

A stochastic formulation

When the number of signaling molecules, N, is small, stochasticity may have a considerable influence on the dynamics of the cascade. To describe the stochastic dynamics of the cascade, we adopt the chemical master equation (45) as

$$\frac{dP(\mathbf{n}, t)}{dt} = \sum_k (W_k(\mathbf{n} - \mathbf{b_k})P(\mathbf{n} - \mathbf{b_k}, t) - W_k(\mathbf{n})P(\mathbf{n}, t)), \qquad (2)$$

where $\boldsymbol{n} = (n_1, \ldots, n_M)$. In Eq. 2, $P(\boldsymbol{n}, t)$ is the probability that the number of molecules is $\boldsymbol{n}$ at time t, and $\boldsymbol{b_k}$ is a vector of the changes in $\boldsymbol{n}$ induced by the k^{th} reaction. For odd k, $\boldsymbol{b_k} = (0,\ldots, 0, 1, 0,\ldots, 0)^{\mathbb{T}}$, where all elements except that of $(k + 1)/2^{\text{th}}$ are zero, and $\mathbb{T}$ represents the transpose of a vector, and for even k, $\boldsymbol{b_k} = -\boldsymbol{b_{k-1}}$. The value $W_k(\boldsymbol{n})dt$ is the transition probability such that the k^{th} reaction occurs in the next time interval dt, provided that the number of molecules is $\boldsymbol{n}$. The value $W_k(\boldsymbol{n})$ is assumed to be time-invariant. The $(2i - 1)^{\text{th}}$ and $(2i)^{\text{th}}$ reactions, respectively, correspond to the activation and inactivation reactions of the i^{th} signaling molecules. The value $W_k(\boldsymbol{n})$ is defined as

$$W_k(n) = \begin{cases} k_{f_1}I(t)(N - n_1) & \text{if } k = 1 \\ k_{b_1}n_1 & \text{if } k = 2 \\ k_{f_i}n_{i-1}(N - n_i)/N & \text{if } k = 2i - 1 \quad \text{for } i \geq 2. \\ k_{b_i}n_i & \text{if } k = 2i \quad \text{for } i \geq 2 \end{cases} \qquad (3)$$

To describe the transient dynamics of the cascade we use $x_i(t)$, rather than $n_i(t)$, because we will compare the dynamics of the cascade for different values of N. As defined before, we use the lower-case x_i to designate a nonrandom value of the ratio of active molecules in the i^{th} step. In contrast, the upper-case X_i represents the random variable of the ratio of active molecules in the i^{th} step.

Furthermore, we use the following variables to characterize the stochastic behaviors of the cascade:

$P(X_i = x_i, t)$: the marginal probability that $X_i = x_i$ at t;
$P_{X_i=0}$: the marginal probability that $X_i = 0$ for all $t \in (0, \infty)$; and
$P_{X_i>0}$: the marginal probability that $X_i > 0$ for at least one $t \in (0, \infty)$ (so $P_{X_i>0} = 1 - P_{X_i=0}$).

In this analysis, we focus on transient behaviors of the signaling cascade. Although various patterns of transient inputs exist, we select a binary input with duration τ for simplicity. This choice does not lack biological validity since inputs to cells can often be regarded as binary approximately. Specifically, we assume that all signaling molecules are inactive for $t \in (-\infty, 0)$, and at time $t = 0$ the cascade receives a constant binary stimulation I_0 for a duration τ, i.e., $I(t) = I_0$ for $t \in [0, \tau]$.

Characterization of the transient response of the cascade

For each step of the cascade, we introduce the following four characteristics of the transient response:

1. Signal integral, $\mathcal{I}(N,i,\tau)$: the temporally integrated response of $X_i(t)$, i.e., $\mathcal{I}(N,i,\tau) = \int_{t=0}^{\infty} dt X_i(t)$.
2. Signal amplitude, $\mathcal{A}(N,i,\tau)$: the maximal response of $X_i(t)$, i.e., $\mathcal{A}(N,i,\tau) = \max_{t\in(0,\infty)} X_i(t)$.
3. Signaling time, $\mathcal{T}(N,i,\tau)$: the time at which X_i reaches its maximum (defined only when the signal amplitude is greater than zero).
4. Signal duration, $\mathcal{D}(N,i,\tau)$: the signal integral over the signal amplitude, i.e., $\mathcal{D}(N,i,\tau) = \mathcal{I}(N,i,\tau)/\mathcal{A}(N,i,\tau)$ (defined only when the signal amplitude is greater than zero).

N, i, and τ are the number of molecules, the cascade step, and the input duration, respectively. These characteristics are schematically represented in Fig. 2 *A*. Since these variables vary for each sample path of the cascade, they are random variables.

To compare the responses of the stochastic and deterministic models, we use the relative values of the characteristics defined as the ratio of the stochastic values to their deterministic counterparts obtained from Eq. 1. Thus, for any given N, the relative signal integral, the relative signal amplitude, the relative signaling time, and the relative signal duration are defined respectively as $R_{\mathcal{I}}(N,i,\tau) = \mathcal{I}(N,i,\tau)/\mathcal{I}^{\rm det}(i,\tau)$, $R_{\mathcal{A}}(N,i,\tau) = \mathcal{A}(N,i,\tau)/\mathcal{A}^{\rm det}(i,\tau)$, $R_{\mathcal{T}}(N,i,\tau) = \mathcal{T}(N,i,\tau)/\mathcal{T}^{\rm det}(i,\tau)$, and $R_{\mathcal{D}}(N,i,\tau) = \mathcal{D}(N,i,\tau)/\mathcal{D}^{\rm det}(i,\tau)$, where $\mathcal{I}^{\rm det}(i,\tau)$, $\mathcal{A}^{\rm det}(i,\tau)$, $\mathcal{T}^{\rm det}(i,\tau)$, and $\mathcal{D}^{\rm det}(i,\tau)$ are deterministic counterparts of the signal integral, the signal amplitude, the signaling time, and the signal duration that are defined as $\int_{t=0}^{\infty} dt x_i(t)$, $\max_{t\in(0,\infty)} x_i(t)$, the time at which x_i reaches its maximum, and $\mathcal{I}^{\rm det}(i,\tau)/\mathcal{A}^{\rm det}(i,\tau)$, respectively.

The averages of the first two relative variables are designated by $\langle R_{\mathcal{I}}(N,i,\tau)\rangle$ and $\langle R_{\mathcal{A}}(N,i,\tau)\rangle$. Since the signaling time and the signaling duration can be defined only when the signal amplitude is greater than zero, the conditional averages $\langle R_{\mathcal{T}}(N,i,\tau)\rangle_c$, and $\langle R_{\mathcal{D}}(N,i,\tau)\rangle_c$ are used to evaluate the transient responses, where the condition is $X_i > 0$ for at least certain $t\in(0,\infty)$.

The signaling time, the signal duration, and the signal amplitude we use in this work are closely related to those used by Heinrich et al. (20), which can specifically be extended to the stochastic versions as $\tilde{\mathcal{T}}(N,i,\tau) \equiv \int_{t=0}^{\infty} t X_i(t)/\mathcal{I}(N,i,\tau)dt$, $\tilde{\mathcal{D}}(N,i,\tau) \equiv \sqrt{\int_{t=0}^{\infty} t^2 X_i(t)/\mathcal{I}(N,i,\tau)dt - \tilde{\mathcal{T}}(N,i,\tau)^2}$, and $\tilde{\mathcal{A}}(N,i,\tau) \equiv \mathcal{I}(N,i,\tau)/2\tilde{\mathcal{D}}(N,i,\tau)$. Since we observed no qualitative differences in our results when using these definitions, we only use $\mathcal{A}(N,i,\tau)$, $\mathcal{T}(N,i,\tau)$, and $\mathcal{D}(N,i,\tau)$ in the following analysis.

Numerical methods

For numerical calculations of the time series of X_i(t) and the statistics of the characteristics defined above, we use Gillespie's algorithm, a Monte Carlo method to numerically calculate sample paths obeying the chemical master equation (46). The statistics of the characteristics are calculated from 10,000 independent samples.

Reaction rate constants

Reaction rate constants are set as follows: $k_{f_1} = 1/(2K_D)$, $k_{b_1} = 1/2$, $k_{f_i} = (1+K_D)/(2K_D)$ $(i \geq 2)$, and $k_{b_i} = 1/2$ $(i \geq 2)$, where $K_D = k_{b_1}/k_{f_1}$ is the dissociation constant in the first step. According to these settings, if K_D is fixed to a constant value, the average responses at all steps to a stationary input with $I_0 = 1$ are the same regardless of the number of molecules N. The results in the following analysis are obtained mainly with $K_D = 1$, but we also examine some cases with different values of K_D.

Input signal

The binary input amplitude I_0 is fixed at one for all simulations. The input duration τ is calculated so that the maximum activity of the first step of the

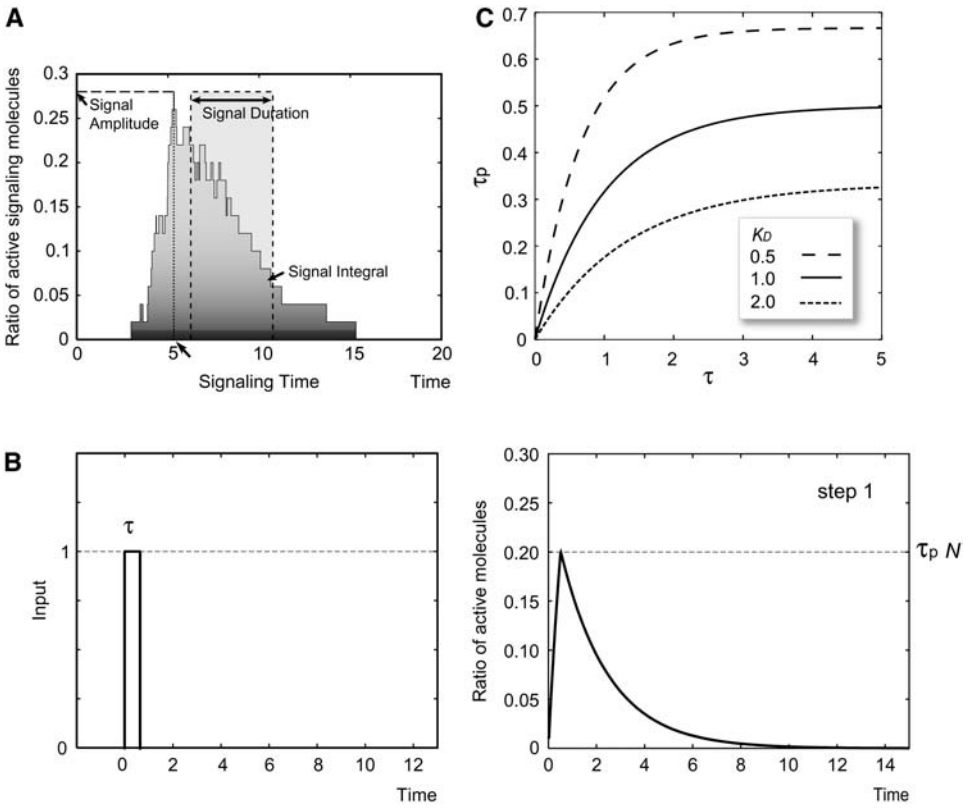

FIGURE 2 (*A*) The definitions of the signal integral, the signal amplitude, the signaling time, and the signal duration. The solid line represents a sample path of the fourth step of the cascade for $N = 50$. The signal integral is defined as the area of the sample path, which is designated with dark gradation. The signal amplitude is the maximum ratio of the active signaling molecules. The signaling time is defined as the time at which the maximum is reached. The signal duration is the width of a rectangle whose area and height are, respectively, equal to the signal integral and the signal amplitude. (*B*) The relation between τ and τ_p. The value τ is calculated so that the maximum activity of the first step of the cascade in the deterministic case becomes $\tau_p N$. The left figure is the time-series of an input and the right one is the response in the first step of the cascade to the input. (*C*) The relation between τ and τ_p for different values of K_D, where $\tau = -\log(1-(1+K_D)\tau_p)/(k_{f1}+k_{b1})$.

cascade in the deterministic case (see Eq. 1) is $\tau_p N$ ($\tau_p \in [0,1]$) (see Fig. 2 *B*). Thus, $\tau = -\log(1 - (1 + K_D)\tau_p)/(k_{f1} + k_{b1})$. We use τ_p as a control parameter of the input intensity rather than τ because τ_p has a more intuitive meaning than τ (see Fig. 2 *C*). Accordingly, the characteristics of the transient response such as $\mathcal{I}(N,i,\tau)$ are rewritten as $\mathcal{I}(N,i,\tau_p)$.

In the next section, we first examine the relation between the signal amplitude and the number of signaling molecules N for different input intensity τ_p. Second, we show that a strong input with large τ_p and a weak input with small τ_p can be discriminated through the cascade when the number of molecules N is small. Since the value of τ_p determines the activity of the first step of the cascade as defined above, we interpret an input with large τ_p as a true signal, and an input with small τ_p as an error signal.

RESULTS

An optimal number of molecules for signal amplification

Fig. 3 shows typical sample paths obtained by numerical simulations for $N = 50$ and $N = 10{,}000$ with $\tau_p = 0.2$. The behavior of the cascade changes with N, because the stochasticity in the dynamics depends strongly on the number of molecules. For $N = 50$, the probability that the response is more amplified than the corresponding deterministic solution obtained from Eq. 1 is high. For $N = 10{,}000$, the shapes of the stochastic response hardly change among sample paths, and almost coincides with the deterministic one.

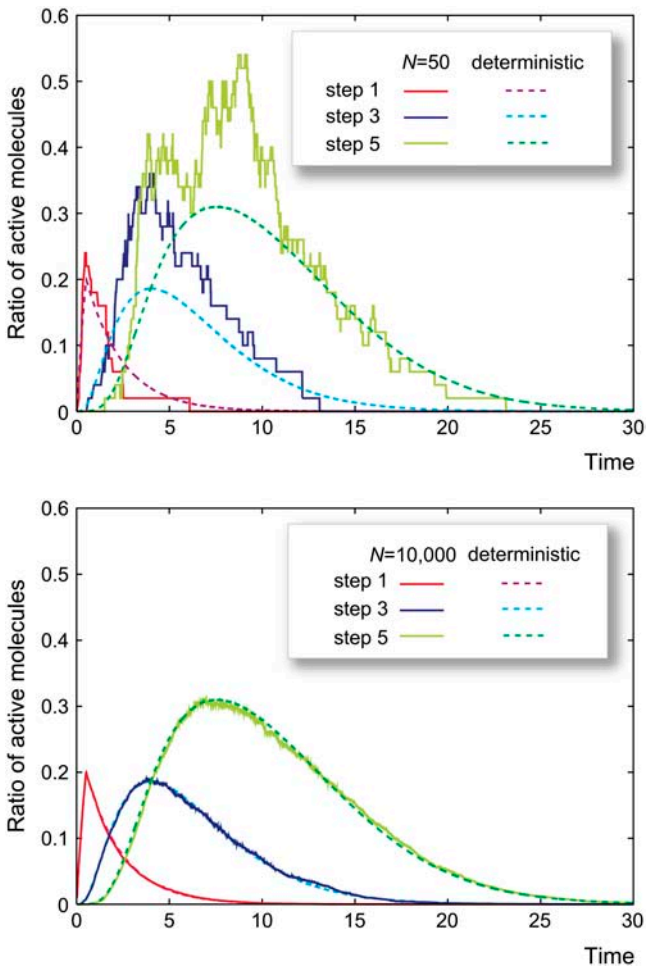

FIGURE 3 Sample paths (*solid lines*) of responses of the cascade for $N = 50$ and $N = 10{,}000$ at steps 1, 3, and 5 with corresponding deterministic solutions (*dashed lines*). The amplification of signals and the truncation of the tails of the responses are observed for $N = 50$.

We quantify this amplification of the response for small N by the average of the relative signal amplitude, $\langle R_{\mathcal{A}}(N,i,\tau_p)\rangle$. For the first step of the cascade, $\langle R_{\mathcal{A}}(N,i,\tau_p)\rangle$ is monotonically decreasing with the increase in N, as shown in Fig. 4. The amplification of $\langle R_{\mathcal{A}}(N,i,\tau_p)\rangle$ can be attributed to the fluctuations originating from the small number of the signaling molecules, since $R_{\mathcal{A}}(N,i,\tau_p)$ is defined as the relative maximal response. When $N = 10$, the probability that all N molecules of the first step become active is significant. However, this probability decreases as N increases, and is negligible for $N = 10{,}000$. As a result, the average of the signal amplitude $\langle R_{\mathcal{A}}(N,i,\tau_p)\rangle$ of the first step decreases monotonically as N increases.

Interestingly, however, the curves of $\langle R_{\mathcal{A}}(N,i,\tau_p)\rangle$ become bell-shaped for $i \geq 2$. This indicates that each cascade step has an optimal number of molecules N_i^{opt} for the signal amplification when $i \geq 2$. The value of N_i^{opt} depends both on the cascade step i and the input intensity τ_p. This emergence of an optimal number of molecules can be attributed to the interplay between the probability of signal loss and that of signal amplification. As discussed for $i = 1$, the fluctuations originating from the small number of molecules are the driving force of the signal amplification. However, if the signal is not detected accidentally at a certain cascade step $i > 1$, that is, $X_i = 0$ for all $t \in (0,\infty)$, the activity of the all steps downstream of the i^{th} step becomes 0. Mathematically, the signal loss at the cascade step $j > 1$ is defined as $X_i = 0$ for all $t \in (0,\infty)$ holds at a certain cascade step $i \leq j$. Thus, large fluctuations due to the small number N also increase the

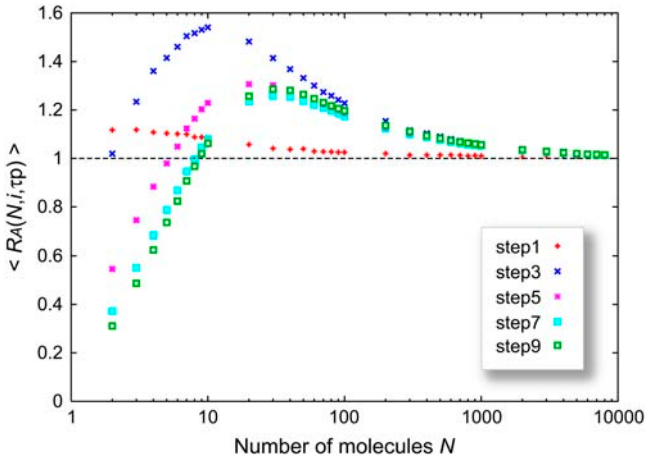

FIGURE 4 The average relative signal amplitude $\langle R_{\mathcal{A}}(N,i,\tau_p)\rangle$ as a function of N for different steps of the cascade. The value τ_p is set to be 0.2.

risk of signal loss through the cascade. This risk decreases as N increases, but the driving force for the signal amplification also decreases as N increases, as reflected in the convergence for large N of the average signal amplitude to that obtained from the deterministic model, i.e., $\lim_{N\to\infty}\langle R_{\mathcal{A}}(N,i,\tau_p)\rangle = 1$. Thus, the emergence of the optimal number of molecules N_i^{opt} for the signal amplification is a consequence of the balance between the reliability of signal propagation and the amplification of signals by fluctuations. In addition, for higher step numbers i, the risk of signal loss is greater and consequently the peak of the $\langle R_{\mathcal{A}}(N,i,\tau_p)\rangle$ curve shifts to the right as shown in Fig. 4. This risk of signal loss is further investigated in the next section.

In the above analysis, the input intensity τ_p is fixed at $\tau_p = 0.2$, but we can observe the emergence of a peak in the $\langle R_{\mathcal{A}}(N,i,\tau_p)\rangle$ curve for $i \geq 2$ for different values of τ_p. Fig. 5 shows the $\langle R_{\mathcal{A}}(N,i,\tau_p)\rangle$ curves for several τ_p values at cascade steps 3, 5, 7, and 9. The position of the peak shifts to the right as τ_p decreases. This is attributed to the decrease in the average number of activated molecules as τ_p decreases. Thus, the probability of signal loss becomes high, which leads to the shift of the peak to the right.

Interestingly, we find that, for each cascade step all the $\langle R_{\mathcal{A}}(N,i,\tau_p)\rangle$ curves for different τ_p seem to intersect at one point. This suggests the existence of a threshold value of N, N_θ, below which $\langle R_{\mathcal{A}}(N,i,\tau_p)\rangle$ for a stronger input becomes larger, while $\langle R_{\mathcal{A}}(N, i, \tau_p)\rangle$ converges to 1 regardless of τ_p for $N \gg N_\theta$. Therefore, when $N < N_\theta$ and a cell receives two inputs with different intensities, the ratio of the average output for the stronger input to that for the weaker one becomes larger compared to deterministic situations. The value of N_θ increases almost exponentially as the cascade step i increases (see Fig. 6). In other words, the range of N that satisfies $N < N_\theta$ becomes wider for larger i. The same tendency is seen for different values of K_D, as shown in Fig. 6.

An optimal number of molecules for signal discrimination

We have evaluated the influence of stochasticity in terms of the average response of the cascade. However, the average response of a cell provides us with only partial information on each response. Thus, more detailed analysis on each response is required when the output of the cascade is critical to the fate of the single cell. One of the most important properties revealed by focusing on the behavior of each response is the probability that signals are lost through the cascade. This signal loss is attributed not only to the stochasticity originating from finite N but also to the fact that $N = 0$ is an absorbing boundary.

The probability of signal loss largely depends on the input intensity τ_p. The probability is high when τ_p is small and vice versa. This suggests a capability in the signaling cascade to actively discriminate true signals with large τ_p from error signals with small τ_p in a probabilistic manner. To quantitatively evaluate the performance of the signal discrimination by the cascade, we use the probability of the signal loss, $P_{X_i=0}$. Since $P_{X_i=0}$ cannot be zero for finite N, the evaluation of the signal discrimination function requires comparison between $P_{X_i=0}$ for true signals and $P_{X_i=0}$ for error signals. For this comparative evaluation, we introduce concepts of false-positive and false-negative errors (47).

The false-positive error in this article means that the cascade responds to error signals. The probability that this

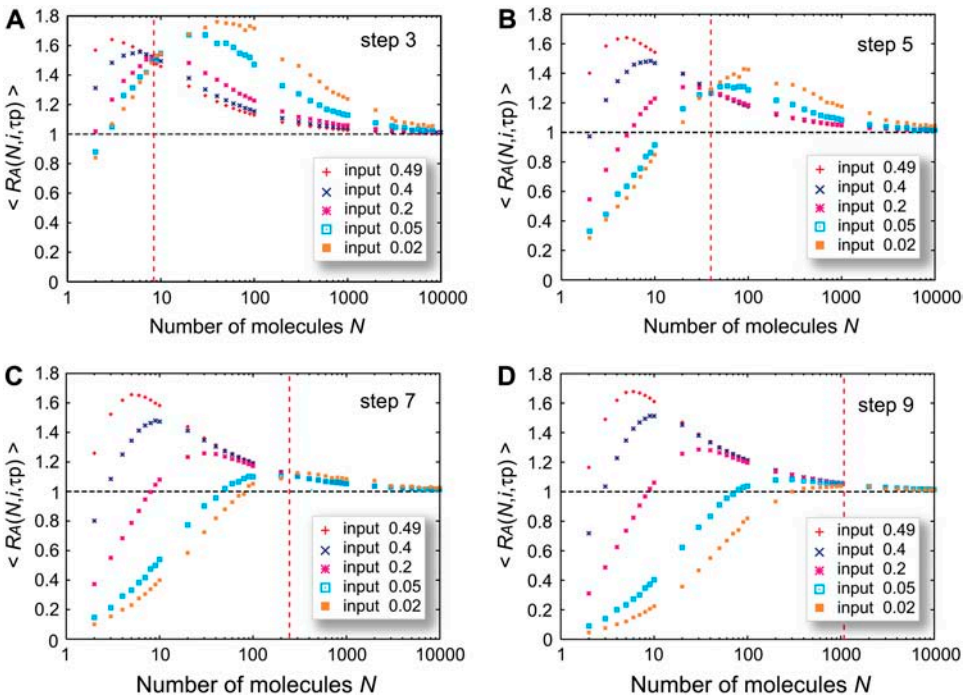

FIGURE 5 The $\langle R_{\mathcal{A}}(N,i,\tau_p)\rangle$ curves for different values of the input intensity τ_p at cascade steps (*A*) 3, (*B*) 5, (*C*) 7, and (*D*) 9.

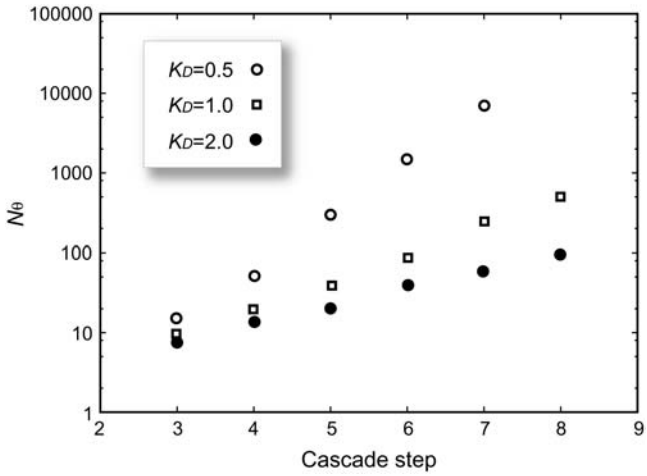

FIGURE 6 The relation between N_θ and the cascade step i for different K_D values. The value N_θ increases exponentially with i.

error occurs is $P^{FPE} \equiv P_{X_i>0}$ for error signals. In a sense, a cascade with a low false-positive error is ''specific'' because the cascade responds exclusively to the true signals. In contrast, the false-negative error means that the cascade fails to respond to true signals. The probability that this error occurs is $P^{FNE} \equiv P_{X_i=0}$ for true signals. A cascade with a low false-negative error is viewed to be ''reliable'' because the cascade can detect true signals with high probability. The values P^{FPE} and P^{FNE} are negatively correlated, as shown in Fig. 7, where τ_p values for true and error signals are 0.2 and 0.01, respectively. In other words, the specificity of the signaling cascade trades off with the reliability of the cascade. As a result, the evaluation of the total performance of a cascade depends on a function required for that cascade.

To incorporate a biased requirement for the cascade into the evaluation, we introduce an indicator of signal discrimination $D = (1 - P^{FPE})^a(1 - P^{FNE})^b$. Larger D means the higher degree of the signal discrimination. Therefore, if the exponent a is large, then the reduction of the false-positive error is preferred. In contrast, if the exponent b is large, the reduction of the false-negative error is favored. By plotting D as a function of N for various values of a and b, we observe that it has a peak for N being several decades, as shown in Fig. 8. This property indicates that there exists an optimal number of signaling molecules for each cascade step with respect to the signal discrimination. As expected, the peak shifts to the left when a becomes large, because large a biases the requirement of the cascade to reduce the false-positive error, while the peak shifts to the right when b becomes large because large b places a disproportionate emphasis on reduction of the false-negative error. Similar phenomena can be observed for different values of K_D and τ_p.

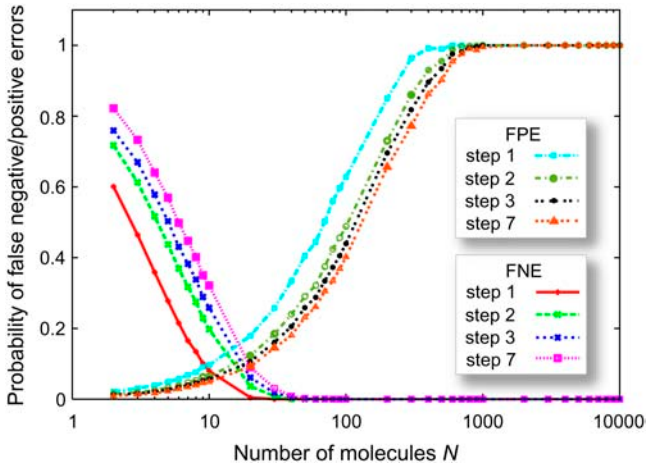

FIGURE 7 The probability of the false-positive error P_{FPE} for error signals with $\tau_p = 0.01$ and the false-negative error P_{FNE} for true signals with $\tau_p = 0.2$ at the cascade steps 1, 2, 3, and 7.

Enhancement of the reliability and the specificity of the signal transduction pathway can be a crucial process for cells. Several biological mechanisms such as high sensitivity for signals, checkpoint, and kinetic proofreading mechanisms have been proposed for enhancement of specificity (47). Reliability may be enhanced by redundant architectures of signal transduction pathways and few sequential reactions (47).

While these mechanisms relate specific network structures or detailed chemical processes to the functions of the signal discrimination, none of them are related to the number of signaling molecules. Our results indicate that the number of signaling molecules can be an important control parameter for the signal discrimination. Furthermore, by introducing the concepts of the false-negative and false-positive errors, we have shown that the specificity and the reliability of the cascade can be evaluated quantitatively. Since systems biology places great emphasis on quantification of intracellular phenomena, the mathematical formulation used in this work may provide us with another possible interpretation of experimentally quantified properties of pathways.

Other properties

In addition to signal amplification and discrimination, there are other properties that are acquired by a cascade when the number of signaling molecules is small. The first property is a quicker response. Fig. 9 *A* shows the relation between the average of the relative signaling time $\langle R_T(N, i, \tau_p)\rangle_c$ and the number of molecules N. For smaller N, $\langle R_T(N, i, \tau_p)\rangle_c$tends to be smaller than one. This property means that a quicker response can be achieved for smaller N, which is usually a desirable property of signal transduction pathways.

Another property is shorter response duration. Fig. 9 *B* shows the relation between the average of the relative signal duration $\langle R_D(N, i, \tau_p)\rangle_c$ and N. $\langle R_D(N, i, \tau_p)\rangle_c$is a monotone increasing function with N. This is because the tail of the response tends to be truncated for small N, which is also a result of the stochasticity and the absorbing boundary at $N = 0$. Although short signal duration is regarded as a desirable

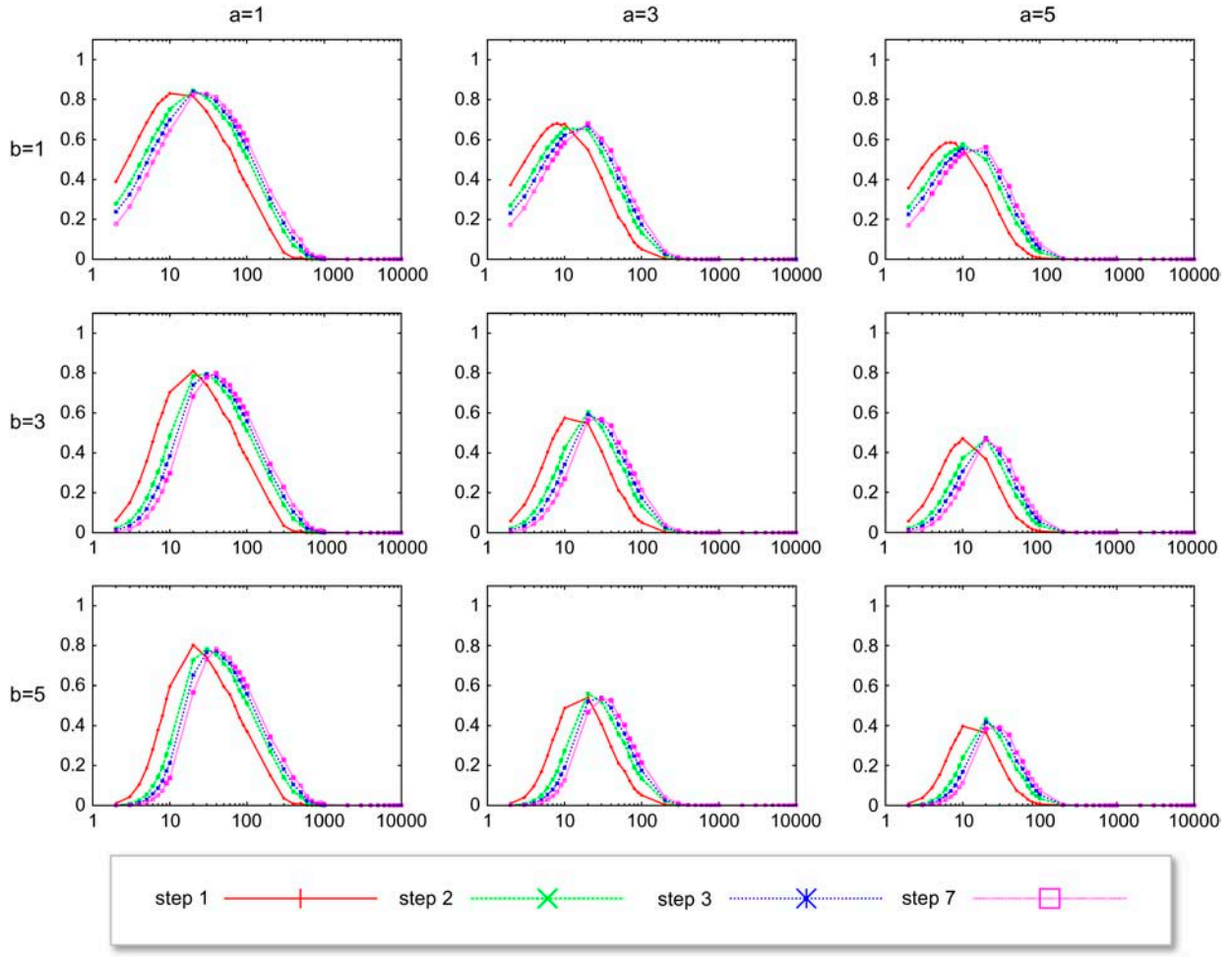

FIGURE 8 The indicator of signal discrimination $D \equiv (1 - P_{\mathrm{FPE}})^{a}(1 - P_{\mathrm{FNE}})^{b}$ as a function of N for different values of a and b. The vertical and horizontal axes in each figure indicate the value of D and the number of molecules N, respectively. Red curves with crosses, green curves with crosses, blue curves with asterisks, and purple curves with open boxes represent the values of D for the first, the second, the third, and the seventh steps of the cascade, respectively.

property in some cases (20), we have no standard criterion by which to evaluate this. On the one hand, this property may enhance the ability of the downstream cascade to distinguish consecutive multiple inputs; on the other hand, the shortened signal duration may reduce the probability of the detection by downstream reactions. The requirement for short or long signal duration strongly depends on properties in the downstream reactions. Thus, this result just indicates that N can be a control parameter for the signal duration.

SUMMARY AND DISCUSSION

Summary of results

In this study, we have examined the performance of signal transduction in a signaling cascade by focusing on the influence of three parameters: the number of signaling molecules N, the input intensity τ_{p}, and the cascade step i. We have found that each step of the signaling cascade has an optimal number $N_{\mathrm{i}}^{\mathrm{opt}}$ of molecules at which the average signal amplitude of the response is maximized for $i \geq 2$. This optimal number $N_{\mathrm{i}}^{\mathrm{opt}}$ was shown to be a consequence of the balance between the failure of signal propagation by signal loss and the signal amplification by fluctuations in stochastic reactions. In addition, we have demonstrated that the cascade step i and the input intensity τ_{p} are two important control parameters for the determination of the value of $N_{\mathrm{i}}^{\mathrm{opt}}$.

We have also shown that a small number of signaling molecules endows the cascade with an ability to actively discriminate true signals and error signals. A tradeoff relation is found between reliable signal transduction with low false-negative errors and specific signal transduction with low false-positive errors. In addition, we have shown that the specificity and the reliability are balanced for an optimal N even if a biased requirement for either the specificity or the reliability is imposed. Furthermore, several properties of the cascade such as the signal duration and the signaling time are strongly influenced by the stochasticity originating from the small number of signaling molecules. By a detailed analysis of the probability of signal loss, we have clarified that the

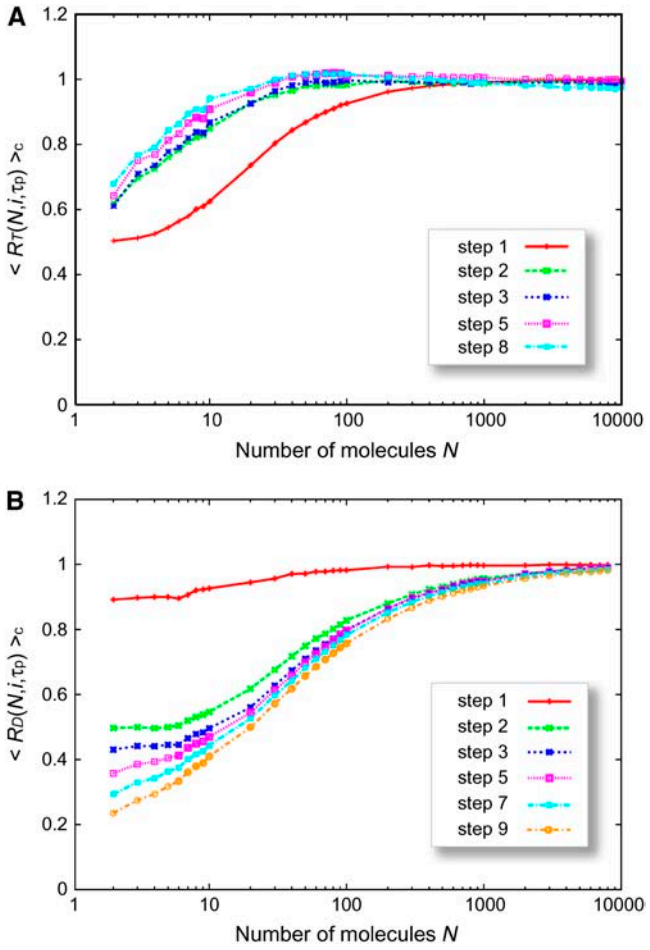

FIGURE 9 (*A*) The average of the relative signaling time $\langle R_{\mathcal{T}}(N,i,\tau_{\mathrm{p}})\rangle_{\mathrm{c}}$ as a function of N for different steps of the cascade. The value τ_{p} is set to be 0.2. (*B*) The average of the relative signal duration $\langle R_{\mathcal{D}}(N,i,\tau_{\mathrm{p}})\rangle_{\mathrm{c}}$ as a function of N for different steps of the cascade. The value τ_{p} is set to be 0.2.

mechanism of these phenomena is the interplay between stochasticity due to the small number of N and the absorbing boundary at $N = 0$. These results have several biological and mathematical implications.

Biological implications

The small number of molecules is often viewed as a major source of disturbance to reliable functioning of intracellular networks due to the stochasticity it generates (48), but it has a strong biological advantage from the viewpoint of energetics. Since synthesis and degradation of molecules are usually associated with energy consumption, cells need to consume energy to maintain a large number of molecules. In addition, each phosphorylation reaction required for activation of signaling molecules also consumes ATP. Thus, activating more signaling molecules requires more energy. This energetic requirement may impose an evolutionary force for the small number of molecules in cells. The extremely small number of lactose repressor may be an indication of such evolutionary force (49,50). Consequently, the actual number of signaling molecules is usually expected to be determined by the balance between this energetic requirement and reliable signal processing facilitated by a large number of molecules. However, according to our results, some signal processing abilities such as signal amplification and signal discrimination can be enhanced by a small number of signaling molecules. Therefore, we suggest that the enhancement may be another evolutionary force for the selection of processing with the small number of intracellular signaling molecules.

The order of the total number of proteins in actual cells widely ranges, e.g., from 10 to 10^6 in yeast (51). Compared with this experimental data, the optimal number of signaling molecules shown in this work seems to be small. However, intracellular molecules are not distributed homogeneously in a cell and all reactants in a cell do not always participate in a reaction (52,53). Rather, intracellular reactions occur heterogeneously in a cell. This heterogeneity is expected to be facilitated by slow diffusion in a cell (54,55). In addition, localization by anchor proteins, scaffold proteins, or compartmentation may control the spatial organization of intracellular reactions (56–59). When the number of signaling molecules participating in a signal transduction is restricted by this spatial heterogeneity, the effective number of molecules may be far below the total number of molecules in a cell, and our results may be interpreted as the local phenomena in cells. Since the study of spatial orchestration of intracellular reactions is in an early phase of theoretical as well as experimental investigation (55,60), our result provides a theoretical clue for future research. Furthermore, the probabilistic transduction of signals shown in this article may be linked to more complicated biological processes.

Evidence of the heterogeneous responses of cells to stimuli has been accumulating recently. While the origin of such heterogeneous responses is typically attributed to the stochastic activations of genes, our result suggests that the probabilistic transmission of signals by signaling cascades can be another source of the heterogeneity. Since it is still experimentally difficult to discriminate the origins of the heterogeneity, it is indispensable to include the probabilistic transmission of signals in the list of candidates for the origins of the heterogeneity. From the experimental viewpoint, this could be indirectly tested by simultaneously observing the responses of the target genes and the activities of promoters that are the first receivers of the extranuclear signals transmitted by signal transduction pathways.

One issue that is not addressed in this article is the influence of variability in the number of signaling molecules itself due to gene expression. Since the timescale of signal transduction is typically much faster than that of the change in the numbers of molecules through gene expression, the numbers of each kind of molecule can be regarded as constant during signal transduction. However, this number

can be distributed around N. As demonstrated in this article, the number of signaling molecules is a key control parameter for signal amplification and discrimination abilities. Thus, it may be possible that the number of signaling molecules is regulated so that it achieves the optimal performances of the signal amplification and discrimination for given conditions. However, this controllability of the performance of the signaling cascade by the number of molecules entails the sensitivity to the fluctuations of the number of molecules. Although we have confirmed that small variations in the number of signaling molecules have little influence on our results, the detailed relation between the controllability of the performances and the robustness to the fluctuations in the number of each kind of signaling molecule is an important future problem.

Mathematical implications

From the mathematical viewpoint, the results shown in this article can be attributed to the interplay between two different effects of the finite number of signaling molecules. One is the stochastic occurrence of intracellular chemical reactions originating from the finite number of reactants. The other is the absorbing boundary at $N = 0$, which is due to the architecture of the signaling cascade. Neither of these effects is sufficient individually. In that respect, the phenomena shown in this study are fundamentally different from well-known stochastic phenomena such as stochastic resonance.

One of a few important studies where both the stochasticity and the absorbing boundary are considered addresses the stationary distribution of the plasmid copy number. When the plasmid copy number is small, one of the daughter cells cannot receive a copy of the plasmids from the mother cell during cell division (61). Another study addresses the discreteness-induced state transitions in autocatalytic cycles, and shows the emergence of new states induced by discrete numbers of components (62). In contrast to these works, we have focused on how the transient dynamics of the signaling cascade and the influence of the small number of signaling molecules can enhance the performance of information processing.

The influence of the boundary condition at $N = 0$ analyzed in this article cannot be handled by the simple linearization methods of stochastic chemical reactions, which have been intensively investigated (63–65). Since most intracellular reactions are highly nonlinear and the existence of such boundary conditions in intracellular reactions is prevalent when the number of molecules is small, there is a need to go beyond these linear descriptions in order to elucidate profound roles of stochasticity in cellular activities.

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